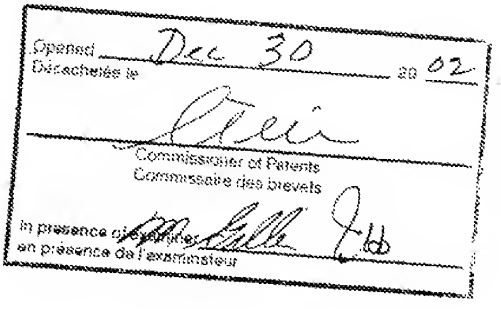
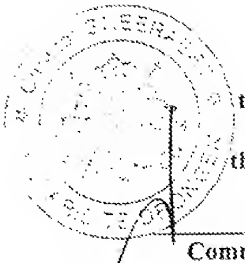
MINUTES OF SCIENTIFIC BOARD

MARCH 27,28 1981

March 27th, morning, Battelle Auditorium

2. Research reports





This is EXHIBIT FIERS-29
to
the Affidavit of Walter C. Fiers
sworn before me
this 19 th day of November, 2001

Commissioner for Oath or Notary Public

g. W. Fiers chronicled his laboratory'progress with fibroblast interferon cloning and expression. Different constructions involving λC_{I857} induction have been made, yields are low. Some semisynthetic constructions are progressing but there are no results on expression at the moment. These involve manipulation of the Shine-Dalgarno sequence and it was pointed out that one problem of high levels of expression is that the overproduced protein is toxic to the cell; for example, there is evidence that lac z and y are toxic in some cases. (However, we should be able to get intermediate levels ?). Other work in Ghent concerns the fusion of different lengths of ß-lactamase signal sequence before the IF_p gene. Experiments on expression in mammalian cells and the production of hybridomas making monoclonal antibodies to IF_p are in progress.

OPPOSITION TO EUROPEAN PATENT NO. 0 [illegible] 913 OF BIOGEN INC.
SCHERING AKTIENGESELLSCHAFT
APPEAL FILE NO. T0207/94-334

DECLARATION OF DR. MICHAEL HOUGHTON

I, MICHAEL HOUGHTON, declare and state as follows:

1. I am a citizen of the United Kingdom, residing in Danville, California (U.S.A.).

2. I presently hold the position of Director, Non-A Non-B Hepatitis Research at Chiron, Inc., Emeryville, California, where I have been employed since 1982.

3. I obtained a B.Sc. (Honors) in Biological Sciences from the University of East Anglia in 1972. I obtained a Ph.D. in Biochemistry in 1977 from Kings College, University of London.

4. I am an author or co-author of over 150 research papers that have been published in peer reviewed journals. These papers span my work on the interferon gene system, the acetylcholine receptor, the immunoglobulin E gene organization, viral hepatitis C (HCV), and hepatitis delta (HDV). My publications also include work on eukaryote RNA polymerases and transcriptional control. For my contributions to the study of viral hepatitis C, I was a co-recipient of the following awards:

- 1992 Karl Landsteiner Award with Harvey Alter, Daniel Bradley, Qui-Lim Choo, George Kuo and Lacy Overby;
- 1993 Robert Koch Award with Daniel Bradley;
- 1994 William Beaumont Award (American Gastroenterology Association) with Dr. Bradley, George Kuo and Qui-Lim Choo.

A full and comprehensive list of my professional activities, including publications, is set forth in the compilation annexed hereto.

5. From 1972 to 1982 I was employed in the department of Biochemistry and Molecular Genetics of Searle Research and Development, High Wycombe, Buckinghamshire. From 1978 until 1982, I was the project leader of the Human Fibroblast Interferon Genetics project. This project initially involved cDNA cloning, sequencing, and expression of the rare human fibroblast interferon mRNA. Investigations were also performed on the structure of the fibroblast interferon gene within the human chromosome. My group was the first to publish and file a patent application on the partial cDNA sequence and the genomic organization of the fibroblast interferon gene. See, e.g., Houghton et al., Nucl. Acid Res., 8:1913-1931, 1980; Houghton et al., Nucl. Acid Res., 8:2885-2894, 1980; Houghton et al., Nucl. Acid Res., 9:247-266, 1980.

This is EXHIBIT FIERS-30
to
the Affidavit of Walter C. Fiers
sworn before me
this 13th day of November, 2001

Commissioner for Oath or Notary Public

6. Six patent applications were filed by our group in 1980 claiming IFN-β related inventions. The applications were filed on Feb. 6, 1980, Feb. 28, 1980, April 17, 1980, April 24, 1980, May 12, 1980, and Nov. 18, 1980. The first five applications contained IFN-β cDNA sequence information, beginning with a partial sequence in the Feb. 6 application and ending with a completed cDNA sequence disclosed in the May 12, 1980 application. We filed quickly and successively on the cDNA sequencing aspect of the project, in intervals of about three weeks or less. Expression of biogically-active was IFN-β achieved within about two months from determining the complete IFN-β cDNA sequence.

7. I am informed that Biogen is proprietor of European patent 0 041 313 which claims expression of biologically- and immunologically-active interferon in unicellular host cells and that this patent is being opposed by Schering AG. I have read and understood pages 14-21 of Biogen's observations, dated December 21, 1994, in which they contend the skilled worker having the Taniguchi [D2] sequences in hand and attempting to express recombinant IFN-β, would have had serious concerns about the expressibility of the IFN-β DNA sequence in view of its content of hydrophobic amino acids, three cysteine residues (positions 17, 31, and 141), AUA codon for 2 isoleucine residues, an AUG codon at the start of the mature polypeptide, and other similar problems related to protein composition.

8. Prior to June 6, 1980, I knew the complete nucleotide and amino acid sequence of IFN-β, including that it possessed three cysteine residues, hydrophobic amino acids, an N-terminal methionine at the start of the mature protein, and two AUA codons for isoleucine. This specific knowledge of these characteristics of IFN-β's sequence did not deter me from continuing our efforts to express the cDNA encoding it in *E. coli*. Despite this knowledge, we expected that a significant amount of biologically-active IFN-β would be expressible in bacteria.

9. Once we had obtained the complete IFN-β cDNA, its expression in *E. coli* was routine and straightforward. In fact, expression of biologically-active IFN-β was achieved at our very first attempt, without performing any manipulations to overcome any of the so-called problems, e.g., hydrophobicity, odd number of cysteines, or AUA codons, described in Biogen's observations.

DATE: x May 23rd 1996 x M Haughton

CURRICULUM VITAE

MICHAEL HOUGHTON

Age:	44
Date of Birth:	6th February, 1951
Nationality:	UK
Residence:	Permanent resident of USA
Marital Status:	Married (with two children)

Education

1969-1972	B.Sc. (Honors) Biological Sciences University of East-Anglia, Norwich, England
1973-1977	Ph.D. Biochemistry King's College, University of London, England

Posts

1977-1982	Senior Research Investigator - Human interferon genetics Searle Research Laboratories Buckinghamshire, England
1982-present	Director, Non-A, Non-B Hepatitis Research Chiron Corporation 4560 Horton Street Emeryville, California 94608 USA

Honors

Co-recipient of 1991 Karl Landsteiner Award from the American Association of Bloo Banks for Hepatitis C Viral Research
Recipient of the Robert Koch Award from Germany
Recipient of the Williams Beaumont Prize from the American Gastroenterology Associatio
Honoree of the Japanese Medical Congress

Patents

Numerous patents issued in the fields of recombinant human interferons, bacteri expression vectors, Hepatitis C and D viruses.

Publications

Over 150 publications in the fields of gene regulation, human beta interferon and hepatit C and D viruses.

Publications

Transcriptional and translational control in eukaryotes

1. "The purification and properties of hen oviduct Form B DNA-dependent RNA po merase" M. Houghton and R.F. Cox (1974) Nucl. Acids Res. 1, 299-308.
2. "The presence of ovalbumin mRNA coding sequences in multiple restriction fragmen of chicken DNA" M.T. Doel, M. Houghton, E.A. Cook and N.H. Carey (1977) Nuc Acids. Res. 4, 3701-3713.
3. "The interaction and RNA polymerase II from wheat with supercoiled and line plasmid templates" D.M.J. Lilley and M. Houghton (1979) Nucl. Acids. Res. 6, 507-52
4. "The nature of the interaction of nucleosomes with eukaryotic RNA polymerase D.M.J. Lilley, M.F. Jacobs and M. Houghton (1979) Nucl. Acids Res. 7, 377-399.
5. "The Xenopus Oocyte as a Surrogate Secretory System" C.D. Lane, A. Colman, Mohun, J. Morser, J. Champion, I. Kourides, R. Craig, S. Higgins, T.C. James, S.V Applebaum, R.I. Ohisson, E. Pauchas, M. Houghton, J. Matthews and B.J. Mifl (19800 Eur. J. Biochem. 111, 225-235.
6. "Active multi-subunit ACh receptor assembled by translation of heterologous mRNA Xenopus oocytes." K. Sumikawa, M. Houghton, J.S. Emtage, B.M. Richards and E. Barnard (1981) Nature 292, 862-864.
7. "The molecular cloning and characterization of cDNA coding for the a subunit of th acetylcholine receptor." K. Sumikawa, M. Houghton, J.C. Smith, L. Bell, B.M. Richard and E.A. Barnard (1982) Nucl. Acids Res. 10, 5809-5822.
8. "Cloning and sequence determination of the gene for the human Σ immunoglobulin chain expressed in a myeloma cell line." J.H. Kenten, H.V. Molgaard, M. Houghto R.B. Derbyshire, J. Viney, L.O. Bell and H.J. Gould (1982) P.N.A.S. (USA) 79, 666 6665.
9. "A study of the mRNA and genes coding for the nicotinic acetylcholine receptor." Sumikawa, M. Houghton, R. Miledi and E.A. Barnard (1983) in "Cell Surfac Receptors," Ed. P.G. Strange pp. 249-269 (Ellis Horwood Ltd., U.K.).
10. "Molecular genetics of the acetyl choline receptor and its insertion and organization the membrane", E.A. Barnard, M. Houghton, R. Miledi, B.M. Richards, and Sumikawa, Biol. Cell (1982) 45:363.

11. "The amino-terminal sequence of human fibroblast interferon as deduced primers." M. Houghton, A.G. Stewart, S.M. Doel, J.S. Emtage, M.A.W. Eaton, J.C. Smith, T.P. Pate H.M. Lewis, A.G. Porter, J.R. Birch, T. Cartwright and N.H. Carey (1980) Nucl. Acic Res. 8, 1913-1931.
12. "Human interferon gene sequences" M. Houghton (1980) Nature 285, 536.
13. "The complete amino acid sequence of human fibroblast interferon as deduced usir synthetic oligodeoxyribonucleotide primers of reverse transcriptase" M. Houghto M.A.W. Eaton, A.G. Stewart, J.C. Smith, S.M. Doel, G.H. Catlin, H.M. Lewis, T.P. Pate J.S. Emtage, N.H. Carey and A.G. Porter (1980) Nucl. Acids Res. 8, 2885-2894.
14. "The absence of introns within a human fibroblast interferon gene" M. Houghton, I. Jackson, A.G. Porter, S.M. Doel, G.H. Catlin, C. Barber and N.H. Carey (1981) Nuc Acids Res. 9, 247-266.
15. "The cloning and expression of a human fibroblast interferon gene in bacteria." M Houghton, S.M. Doel, G.H. Catlin, A.G. Stewart, A.G. Porter, W.C.A Tacon, M.A.W Eaton, J.S. Emtage and N.H. Carey (1981) Proceedings of the Battelle Internation genetic Engineering Conference, M. Keenberg, Ed. Battelle Seminars and Studie Program.
16. "Biological Properties of Human Interferon Beta 1 Synthesized in Recombina Bacteria." K.G. McCullagh, J.A. Davies, I.S. Sim, K.M. Dawson, G.J. O'Neill, S.M. Doe G.H. Catlin and M. Houghton (1983) J. Interf. Res. 3, 97-111.
17. "Active hybrids formed between human beta and alpha interferons." A.G. Porter, L.I Bell, J.R. Adai, G.H. Catlin, J.M. Clarke, J.A. Davies, K.M. Dawson, R.B. Derbyshir S.M. Doel, L. Dunthorne, M.E. Finlay, J. Hall, M. Houghton, C. Hynes, I.J. Lindley, M.I Nugent, G.J. O'Neill, J.C. Smith, A.G. Stewart, W.C. Tacon, J.H. Viney, N. Warburto P.G. Boseley and K.G. McCullagh (1985) in "The Biology of the Interferon System Eds. Schellekers & Stewart.
18. "Novel modified beta interferons: gene cloning, expression and biological activity bacterial extracts." A.G. Porter, L.D. Bell, J.R. Adai, G.H. Catlin, J.M. Clarke, J.A Davies, K.M. Dawson, R.B. Derbyshire, S.M. Doel, L. Dunthorne, M.E. Finlay, J. Ha M. Houghton, C. Hynes, I.J. Lindley, M.E. Nugent, G.J. O'Neill, J.C. Smith, A.G Stewart, W.C. Tacon, J.H. Viney, N. Warburton, P.G. Boseley and K.G. McCullag (1986) DNA 5, 137-148.

Hepatitis D virus

19. "The structure, sequence and expression of the hepatitis delta (δ) viral genome." K.J Wang, Q.L. Choo, A.J. Weiner, J.H. Ou, R.C. Najarian, R.M. Thayer, G.T. Mullenbac K.J. Denniston, J.L. Gerin and M. Houghton 91986) Nature, 323, 508-514 (1986 Nature, 328, 456 (1987).

20. "The Viroid-like structure of the hepatitis delta genome: synthesis of a viral antigen recombinant bacteria", K-S. Wang, Q-L. Choo, A.J. Weiner, J-H. Ou, K.J. Denniston J.L. Gerin and M. Houghton (1987) in "The hepatitis delta virus and its infection", M Rizzetto, J.L. Gerin, R.H. Purcell, Eds. pp:. 71-82 (Alan Liss Inc., New York).

21. "Hepatitis delta (δ) cDNA clones: Undetectable hybridization to nucleic acids fro infectious Non-A, Non-B hepatitis materials and hepatitis B DNA", A.J. Weiner, K.-S Wang, Q.-L. Choo, J.L. Gerin, D.W. Bradley, and M. Houghton (1987) J. of Medic. Virology 21: 239-247.

22. "A single antigenomic open reading frame of the hepatitis delta virus encodes th epitope(s) of both hepatitis delta antigen polypeptides $p24^{\delta}$ and $p27^{\delta}$", A.J. Weiner, C L. Choo, K-S. Wang, S. Govindarajan, A.G. Redeker, J.L. Gerin, and M. Houghton. of Virology (1988) p. 594-599.

23. "Towards a vaccine for the prevention of hepatitis delta virus superinfection in HB carriers", A. Ponzetto, M. Eckart, N. D'Urso, F. Negro, M. Silvestro, F. Bonino, K.S Wang, D. Chien, Q.-L. Choo, and M. Houghton, Prog. Clin. Biol. Res. (1993) 382:20 210.

24. "The effects of using recombinant vaccinia viruses expressing either large or sma HDAg to protect woodchuck hepadnavirus carriers from HDV superinfection", M.F Eckart, C. Dong, M. Houghton, N. D'Urso, and A. Ponzetto, Prog. Clin. Biol. Re. (1993) 382:201-205.

Non-A, non-B hepatitis/Hepatitis C virus

25. "Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepati genome", Q-L. Choo, G. Kuo, A.J. Weiner, L.R. Overby, D.W. Bradley, and M Houghton, Science (1989) 244:, pp. 359-362.

26. "An Assay for Circulating Antibodies to a Major Etiologic Virus of Human Non-A, Non-I Hepatitis." G. Kuo, Q.-L. Choo, H.J. Alter, G.I. Gitnick, A.G. Redeker, R.H. Purcell, T Miyamura, J.L. Dienstag, M.J. Alter, C.E. Stevens, G.E. Tegtmeier, F. Bonino, M Colombo, W.-S. Lee, C. Kuo, K. Berger, J.R Shuster, L.R. Overby, D.W. Bradley, M Houghton Science (1989) 244:362-364.

27. "Hepatitis C virus antibodies among risk groups in Spain", J.I. Esteban, L. Viladomiu, A Bonzalez, M. Roget, J. Genesca, J. Guardia, R. Esteban, J.C. Lopez-Talavera, J.M Hernandez, V. Vargas, M. Buti, G. Kuo, Q.-L. Choo and Michael Houghton, Lance (1989) p. 294-296.

28. "Anti-Hepatitis C antibodies and non-A, non-B post-transfusion hepatitis in th Netherlands", C.L. Van Der Poel, H.W. Ressink, P.N. Lelie, A. Leentvaar-Kuypers, Q-L Choo, G. Kuo, M. Houghton, (1989) The Lancet, 2:297-298.

29. "Hepatitis C virus HCV: A causative agent of cryptogenic cirrhosis CC amon Cubans", T. Parker, M. de Medina, L. Jeffers, R. Reddy, D. Bradley, E. Schiff, M Houghton, Q.-L. Choo and G. Kuo, Hepatology (1989) 10:685.

30. "Anti-hepatitis C virus in non-A non-B patients responding and non-responding t alpha 2A interferon", G. Saracco, M. Houghton, G. Kuo, Q.-L. Choo, F. Rosina, V Lattore, M.R. Torrani Cerenzia, L. Chiandussi, F. Bonino and M. Rizzetto, J Hepatc (1989) 9:219.

31. "Non-A, non-B hepatitis and antibody to hepatitis C virus." J.W. Mosley, R.D.Aach F.B. Hollinger, C.E. Stevens, L.H. Barbosa, G.J. Nemo, P.V. Holland, W.H. Bancrof H.J. Zimmerman, G. Kuo, Q.-L. Choo, M. Houghton JAMA 263, 1: 77-78

32. "Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellula carcinoma", M. Colombo, Q.-L. Choo, E. Del Ninno, N. Dioguardi, G. Kio, M.F., Donatc M.A., Tommasini and M. Houghton, The Lancet, (1989) p. 1006-1008.

33. "Detection of antibody to hepatitis C virus in prospectively followed transfusion recip ents with acute and chronic non-A, non-B hepatitis", H.J. Alter, R.H. Purcell, J.W. Shih J.C. Melpolder, M. Houghton, Q.-L. Choo and G. Kuo, New England Journal c Medicine (1989) 321: 1494-1500.

34. "A cDNA fragment of hepatitis C virus isolated from an implicated donor of post-trans fusion non-A, non-B hepatitis in Japan. K. Takeuchi, S. Boonmar, T. Katayama, Q.L Choo, G. Kuo, A.J. Weiner, D.W. Bradley, M. Houghton, I. Saito, T. Miyamura. Nuc Acids Res. (1989) 17, 24:10367-10372.

35. "Hepatitis C virus complementary DNA clones isolated from a single healthy carrie who was shown to be an implicated donor of post-transfusion non-A, non-B hepatitis' T. Miyamura, I. Saito, Y. Kubo, K. Takeuchi, S. Boonmar, T. Katayama, G. Kuo, Q.-L Choo and M. Houghton, In: Proceedings of the International Meeting on Non-A, Nor B Hepatitis, Tokyo, 1989, Shikata, T., Purcell, R.H., Uchida, T. eds., Elsevier Scienc Publishers, Amsterdam.

36. "Autoimmune hepatitis is not associated with antibodies to hepatitis C virus (HCV) J.G. McHutchison, G. Kuo, M. Houghton, Q.-L. Choo, A.G. Redeker, Hepatolog (1989) 10:701.

37. "Intrafamilial transmission of hepatitis C virus", H. Kamitsukasa, H. Harada, M. Yakura A. Fukuda, A. Ohbayashi, I. Saito, T. Miyamura, Q.-L. Choo, M. Houghton and G. Kuo Lancet (1989) 2:987.

38. "High prevalence of antibody to hepatitis C virus in patients with primary liver carc noma", M. Colombo, G. Kuo, Q.-L. Choo, M. Houghton, M.F. Donato, M.A. Tommasi S. Bargiggia, A. Piva, E. Del Ninno and N. Dioguardi, Hepatology (1989) 10:700.
39. "High prevalence of antibody to hepatitis C virus in patients with hepatocellular carc noma HCC", M. Colombo, G. Kuo, Q.-L. Choo, M. Houghton, M.A. Tommasini, M.C Rumi, M.L. Dioguardi, M.F. Donato, and E. Del Ninno, J. Hepatol. (1989) 9:20.
40. "The role of hepatitis C virus HCV in chronic liver disease", W.N. Katkov, H. Cody, A. Evans, G. Kuo, Q.-L. Choo, M. Houghton and J.L. Dienstag, Hepatology (198 10:644.
41. "Elevated serum alanine aminotransferase ALT in blood donors: The contribution hepatitis C virus HCV", W.N. Katkov, L.S. Friedman, H. Cody, A.A. Evans, G. Kuo, C.-. Choo, M. Houghton, C.E. Huggins, and J.L. Dienstag, Hepatology (1989) 10:581.
42. "Circulating antibodies to hepatitis C virus HCV: A study of 160 cases of acute an chronic NANB hepatitis", J.G. McHutchison, G. Kuo, M. Houghton, Q.-L. Choo an A.G. Redeker, Hepatology (1989) 10:645.
43. "Hepatitis C HCV associated idiopathic chronic hepatitis and cryptogenic cirrhosis". Jeffers, M. de Medina, F. Hasan, R. Reddy, T. Parker, M. Silva, L. Mendez, E. Schiff M. Houghton, Q.-L. Choo and G. Kuo, Hepatology (1989) 10:644.
44. "Hepatitis C HCV associated hepatocellular carcinoma", F. Hasan, L. Jeffers, M. d Medina, R. Reddy, T. Parker, E. Schiff, M. Houghton, Q.-L. Choo, and G. Kuo Hepatology (1989) 10:608.
45. "Seroepidemiology of hepatitis C virus HCV in selected population", A.A. Evans, H Cody, G. Kuo, Q.-L. Choo, M. Houghton, W.N. Katkov and J.L. Dienstag, Hepatolog (1989) 10:644.
46. "Blood-borne non-A, non-B hepatitis PT-NANB immunohistochemical identification disease and hepatitis C virus-associated antigens", K. Krawczynski, G. Kuo, A Dibisceglie, M. Houghton and D.W. Bradley, Hepatology (1989) 10:580.
47. "Detection of antibody to hepatitis C virus in patients with various chronic live diseases", A.M. De Bisceglie, H. Alter, G. Kuo, M. Houghton and J.H. Hoofnagle Hepatology (1989) 10:581.
48. "Distinction between chronic and self-limited forms of hepatitis C virus infection", A.M Prince, B. Brotman, T. Huima, P. Krauledat, M. Houghton, G. Kuo, Q.-L. Kuo, In International Meeting on Non-A, Non-B Hepatitis, Tokyo, Japan, 1989, Shikata, T Purcell, R.H., Uchida, T. eds. Elsevier Science Publishers, Amsterdam, pp. 7-16.
49. "Discovery of hepatitis C virus and assay of non-A, non-B hepatitis virus", M Houghton, Jikken Igaku (1990) 8:203-206.
50. "Detection of hepatitis C viral sequences in non-A, non-B hepatitis", A.J. Weiner, G Kuo, D.W. Bradley, F. Bonino, G. Saracco, C. Lee, J. Rosenblatt, Q.-L. Choo and M Houghton, The Lancet (1990) 335: 1-3.
51. "Hepatitis C virus: The major causative agent of viral non-A, non-B hepatitis", Q.-L Choo, A.J. Weiner, L.R. Overby, G. Kuo and M. Houghton, British Medical Bulleti (1990) 46:423-441.
52. "Detection of antibody against antigen expressed by molecularly cloned hepatitis virus cDNA: Application to diagnosis and blood screening for posttransfusion hepat itis", T. Miyamura, I. Saito, T. Katayama, S. Kikuchi, A. Tateda, M. Houghton, Q.-L Choo and G. Kuo, Proc. Natl. Acad. Sci. USA (1990) 87:983-987.
53. "Hepatitis C virus antibodies in southern African blacks with hepatocellular carcinoma M.C. Kew, M. Houghton, Q.-L. Choo and G. Kuo, The Lancet (1990) 335: 873-874.

54. "Hepatitis C virus infection in an area hyperendemic for hepatitis B and chronic live disease: The Taiwan experience", D.-S. Chien, G.C. Kuo, J.-L. Sung, M.-Y. Lai, J.-C Sheu, P.-J. Chen, P.-M. Yang, H.-M. Hsu, M.-H. Chang, C.-J. Chen, L.-C. Hann, Q.-L. Choo, T.-H. Wang and M. Houghton, J. Infect. Dis. (1990) 162:817-822.

55. "Hepatitis C antibody and chronic liver disease in haemophilia", M. Makris, F.E Preston, D.R. Triger, J.C.E. Underwood, Q.-L. Choo, G. Kuo and M. Houghton, Lancet (1990) 335:1117-1119.

56. "Epidemiology of hepatitis C virus. A preliminary study in volunteer blood donors", C.E Stevens, P.E. Taylor, J. Pindyck, Q.-L. Choo, D.W. Bradley, G. Kuo and M. Houghton JAMA (1990) 263:49-53.

57. "Hepatitis C HCV infection in hemodialysis units", L. Jeffers, G. Perez, M. de Medina, E Schiff, C. Ortiz-Interian, J. Bourgoignie, C.A. Vaamonde, M. Houghton, Q.-L. Choo and G. Kuo, Kidney Int. (1990) 37:303.

58. "HCV Immunologic and hybridization based diagnostics", A.J. Weiner, M.A. Truett, J. Rosenblatt, J. Han, S. Quan, A.J. Polito, G. Kuo, Q.-L. Choo and M. Houghton, In: Viral Hepatitis and Liver Disease, Hollinger, F.B., Lemon, S.M., Margolis, H.S., eds., William & Wilkins, 1990, Baltimore, MD, 360-363.

59. "The role of hepatitis C virus in non-B chronic liver disease", W.N. Katkov, J.L Dienstag, H. Cody, A.A. Evans, Q.-L. Choo, M. Houghton and G. Kuo, Archives In Med., (1991) 151:1548-1552.

60. "Antibody to hepatitis C virus in the serum of patients with chronic hepatitis", F. Oliver M. Baldi, M.R. Brunetto, G. Saracco, F. Rosina, M.T. Cerenzia, M. Rizzetto, M.L Soranzo, L. Colla, P. Vallauri, G. Verme, G. Kuo, M. Houghton and F. Bonino, Europea J. Gastroenterology & Hepatology (1990) 2:347-350.

61. "Hepatitis C virus (HCV): A relative of the pestiviruses and flaviviruses", M. Houghton K. Richman, J. Han, K. Berger, C. Lee, C. Dong, L. Overby, A. Weiner, D. Bradley, G Kuo and Q.-L. Choo, In: Viral Hepatitis and Liver Disease, Hollinger, F.B., Lemon, S.M Margolis, H.S., eds., Williams & Wilkins, 1990, Baltimore, MD, 328-333.

62. "Detection and mapping of immunologic epitopes expressed by bacterial cDNA clone of the hepatitis C virus", Q.-L. Choo, K. Berger, G. Kuo and M. Houghton, In: Vira Hepatitis and Liver Disease, Hollinger, F.B., Lemon, S.M., Margolis, H.S., eds., William & Wilkins, 1990, Baltimore, MD., 345-346.

63. "Serodiagnosis of hepatitis C viral infection using recombinant-based assays for circulating antibodies to different viral proteins", G. Kuo, Q.-L. Choo, J. Shuster, C. Kuo, K Berger, W. S. Lee, A. Medina-Selby and M. Houghton, In: Viral Hepatitis and Live Disease, Hollinger, F.B., Lemon, S.M., Margolis, H.S., eds., Williams & Wilkins, 199C Baltimore, MD. 347-349.

64. "HCV. Immunologic and hybridization-based diagnostics", A. J. Weiner, M. A. Truett, J. Rosenblatt, J. Han, S. Quan, A. J. Polito, G. Kuo, Q.-L. Choo and M. Houghton, In: Vira Hepatitis and Liver Disease, Hollinger, F.B., Lemon, S.M., Margolis, H.S., eds., William & Wilkins, 1990, Baltimore, MD, 360-363.

65. "Molecular cloning of hepatitis C virus cDNA from plasma of an implicated donor o post-transfusion non-A, non-B hepatitis", S. Boonmar, K. Takeuchi, Y. Kubo, T Katayama, H. Harada, A. Ohbayashi, Q.-L. Choo, G. Kuo, M. Houghton, I. Saito and T Miyamura, In: Viral Hepatitis and Liver Disease, Hollinger, F.B., Lemon, S.M., Margolis H.S., eds., Williams & Wilkins, 1990, Baltimore, MD. 371-374.

66. "Early events in hepatitis C virus infection of chimpanzees", Y. K. Shimizu, A. J. Weiner J. Rosenblatt, D. C. Wong, M. Shapiro, T. Popkin, M. Houghton, H. J. Alter and R. H Purcell, Proc. Natl. Acad. Sci. USA (1990) 87:6441-6444.

67. "Direct detection of hepatitis C viral sequences in non-A, non-B hepatitis", A.J. Weiner G. Kuo, D.W. Bradley, F. Bonino, G. Saracco, C. Lee, J. Rosenblatt, and Q.-L. Choo Lancet (1990) 338:1-3.

68. "Impact of blood-donor screening for anti-HCV versus ALT, and cofactors for infectivi of anti-HCV-positive blood", C. L. Van der Poel, H. W. Reesink, P. N. Lelie, M. T Cuijpers, A. Leentvaar,-Kuypers, E. Bakker, P. J. Exel-Oehlers, A. Polito, M. Houghto and W. Schaasberg, In: Viral Hepatitis and Liver Disease, Hollinger, F.B., Lemon, S.M Margolis, H.S., eds., Williams & Wilkins, 1990, Baltimore, MD, 427-430.

69. "Open prospective efficacy trial of anti-HCV screening of blood donors to prevent pos transfusion hepatitis: Interim report of the barcelona PTH study", J. I. Esteban, A González, J. M. Hernández, P. Madoz, E. Muniz, J. Torras, J. Enriquez, J. Buenestado C. Martin-Vega, C. Sánchez, R. Esteban, J. Guardia, M. Houghton and H. J. Alter, In Viral Hepatitis and Liver Disease, Hollinger, F.B., Lemon, S.M., Margolis, H.S., eds Williams & Wilkins, 1990, Baltimore, MD, 431-433.

70. "Blood-borne non-A, non-B hepatitis: Detection and identification of hepatitis C viru and disease-associated antigen HCV Ag in hepatocytes", K. Krawczynski, G. Kuo, A Dibisceglie, D. Bradley, M. Houghton, M. Alter and J. Ebert, In: Viral Hepatitis and Live Disease, Hollinger, F.B., Lemon, S.M., Margolis, H.S., eds., Williams & Wilkins, 199(Baltimore, MD, 434-435.

71. "Use of anti-HCV determinations for diagnosis of chronic HCV infection", A. M. Prince B. Brotman, T. Huima, P. Krauledat, M. Houghton, G. Kuo, Q.-L. Choo, A. Polito, R. E Nelio and M. J. Nelles, In: Viral Hepatitis and Liver Disease, Hollinger, F.B., Lemor S.M., Margolis, H.S., eds., Williams & Wilkins, 1990, Baltimore, MD, 450-455.

72. "Role of hepatitis C virus in hepatocellular carcinoma", T. Miyamura, I. Saito, T Yoneyama, K. Takeuchi, A. Ohbayashi, Y. Watanabe, Q.-L. Choo, M. Houghton and G Kuo, In: Viral Hepatitis and Liver Disease, Hollinger, F.B., Lemon, S.M., Margolis, H.S eds., Williams & Wilkins, 1990, Baltimore, MD, 559-562.

73. "Hepatitis C viral cDNA clones isolated from a healthy carrier donor implicated in pos transfusion non-A, non-B hepatitis", K. Takeuchi, S. Boonmar, Y. Kubo, T. Katayama H. Harada, A. Ohbayashi, Q.-L. Choo, G. Kuo, M. Houghton, I. Saito and T. Miyamura Gene (1990) 91:287-291.

74. "Prevalence of anti-HCV antibody in blood donors in the Tokyo area", J. Watanabe, K Minegishi, T. Mitsumori, M. Ishifuji, T. Oguchi, M. Ueda, E. Tokunaga, E. Tanaka, K Kiyosawa, S. Furuta, T. Katayama, G. Kuo, Q.-L. Choo, M. Houghton and K. Nishioka Vox Sang (1990) 5980-12.

75. "Blood screening for non-A, non-B hepatitis by hepatitis C virus antibody assay", T Katayama, S. Kikuchi, Y. Tanaka, I. Saito, T. Miyamura, Q.-L. Choo, M. Houghton an G. Kuo, Transfusion (1990) 30:374-376.

76. "The putative nucleocapsid and envelope protein genes of hepatitis C virus deter mined by comparison of the nucleotide sequences of two isolates derived from a experimentally infected chimpanzee and healthy human carriers", K. Takeuchi, Y Kubo, S. Boonmar, Y. Watanabe, T. Katayama, Q.-L. Choo, G. Kuo, M. Houghton, I Saito and T. Miyamura, J. Gen. Virol. (1990) 71:3027-3033.

77. "Cloning and sequence analysis of hepatitis B virus variants in non-A and non-B infec tions", D. Kremsdorf, V. Thiers, F. Garreau, H. Duclos, C. Porchon, M. Houghton, F Tiollais and C. Brechot, J Hepatol (1990) 11:36.

78. "Hepatitis C virus infection is associated with the development of hepatocellular carc noma", I. Saito, T. Miyamura, A. Ohbayashi, H. Harada, T. Katayama, S. Kikuchi, Y Watanabe, S. Koi, M. Onji, Y. Ohta, Q.-L. Choo, M. Houghton and G. Kuo, Proc. Nat Acad. Sci. USA (1990) 87:6547-6549.

79. "Hepatitis C-associated hepatocellular carcinoma", F. Hasan, L.J. Jeffers, M. D Medina, K.R. Reddy, T. Parker, E.R. Schiff, M. Houghton, Q.-L. Choo and G. Kuo Hepatology (1990) 12:589-591.

80. Nucleotide sequence of core and envelope genes of the hepatitis C virus genom derived directly from human healthy carriers", K. Takeuchi, Y. Kubo, S. Boonmar, Watanabe, T. Katayama, Q.-L. Choo, G. Kuo, M. Houghton, I. Saito, and T. Miyamur Nuc. Acids Res. (1990) 18:4626.

81. "HCV testing in low-risk population", A.J. Weiner, M.A. Truett, J. Rosenblatt, J. Han, S Quan, A.J. Polito, G. Kuo, Q.-L. Choo, M. Houghton and C. Agius, et al. Lancet (199 336:695.

82. "The relation of hepatitis C antibodies to acute non-A, non-B hepatitis NANBH in pre viously untreated hemophilic patients", M. Makris, M.S. Dewar, F.E. Preston, Q.-L Choo, G. Kuo and M. Houghton, Br J Haematol (1990) 74:44.

83. "HCV infection in autoimmune chronic active hepatitis", S. Magrin, A. Craxi, P. Almasio C. Fabiano, G. Fiorentino, U. Palazzo, G.B. Pinzello, G. Provenzano, L. Pagliaro, M Houghton and J.H. Han, J. Hepatol. (1990) 11:99.

84. "Vertical transmission of hepatitis C virus detected by the polymerase chain reaction" M.M. Thaler, D.W. Landers, D.W. Wara, M. Houghton, G. Veereman-Wauters, R.J Sweet, M. Brauer and J.H. Han, Hepatology (1990) 12:849.

85. "Patients with acute and chronic NANB hepatitis", J.G. McHutchison, G. Kuo, M Houghton, Q.-L. Choo and A.G. Redeker, Hepatology (1990) 12:966.

86. "Antibodies to hepatitis C virus anti-HCV in alcoholic liver disease: An analysis of risk factors", R.J. Leal, J.G. McHutchison, M. Houghton, Q.-L. Choo, G. Kuo, A.G. Redeker Hepatology (1990) 12:881.

87. "The role of hepatitis C virus in chronic liver disease in hemophilia", M. Makris, F.E Preston, D.R. Triger, J.C.E. Underwood, G. Kuo, Q.-L. Choo and M. Houghton, Br Haematol (1990) 74:6.

88. "Antibody to the hepatitis C virus in acute hepatitis and chronic liver diseases i Japan", K. Nishioka, J. Watanabe, S. Furuta, E. Tanaka, H. Suzuki, S. Iino, T. Tsuji, M Yano, G. Kuo, Q.-L. Choo, M. Houghton and T. Oda, Liver (1991) 11:65-70.

89. "A high prevalence of antibody to the hepatitis C virus in patients with hepatocellular carcinoma in Japan", K. Nishioka, J. Watanabe, S. Furuta, E. Tanaka, S. Iino, H Suzuki, T. Tsuji, M. Yano, G. Kuo, Q.-L. Choo, M. Houghton and T. Oda, Cancer (199 67:2.

90. "Hepatitis C virus is a distant relative of the flaviviruses and pestiviruses", Q.-L. Choo J. Han, A.J. Weiner, L.R. Overby, D.W. Bradley, G. Kuo and M. Houghton, In Proceedings of the International Meeting on Non-A, Non-B Hepatitis, Tokyo, Japan 1991, Shikata, T., Purcell, R.H., and Uchida, T. eds., Elsevier Science Publishers Amsterdam, pp. 47-52.

91. "The role of chronic viral hepatitis in hepatocellular carcinoma in the United States" A.M. Di Bisceglie, S.E. Order, J.L. Klein, J.G. Wagoner, M.H. Sjogren, G. Kuo, M Houghton, Q.-L. Choo and J.H. Hoofnagle, Amer. J. Gastroenterology (1991) 86:33 338.

92. "Genetic organization and diversity of the hepatitis C virus", Q.-L. Choo, K.H. Richman J.H. Han, K. Berger, C. Lee, C. Dong, C. Gallegos, D. Coit, A. Medina-Selby, P.J. Barr A.J. Weiner, D.W. Bradley, G. Kuo and M. Houghton, Proc. Natl. Acad. Sci. USA (199 88:2451-2455.

93. "Variable and hypervariable domains are found in the regions of HCV corresponding t the flavivirus envelope and NS1 proteins and the pestivirus envelope glycoproteins" A.J. Weiner, M.J. Brauer, J. Rosenblatt, K.H. Richman, J. Tung, K. Crawford, F. Bonino G. Saracco, Q.-L. Choo, M. Houghton and J.H. Han, Virology (1991) 180:842-848.

94. "Confirmation of hepatitis C virus infection by new four-antigen recombinan immunoblot assay", C.L. van der Poel, H.T.M. Cuypers, H.W. Reesink, A.J. Weiner, S Quan, R. DiNello, J.J.P. van Boven, I. Winkel, D. Mulder-Folkerts, P.J. Exel-Oeniers, V Schaasberg, A. Leentvaar-Kuypers, A. Polito, M. Houghton and P.N. Lelie, Lancs (1991) 337:317-319.

95. "Comparison of first and second generation anti-HCV recombinant immunoblot assa with 5' UTR PCR", H.W. Reesink, C.L. Van der Poel, A.D.D. Plaisier, J.W. Verstraten, N Cuypers, Transfusion (1991) 31:57.

96. "Characterization of the terminal regions of hepatitis C viral RNA: identification of con served sequences in the 5' untranslated region and poly(A) tails at the 3c end", J.H Han, V. Shyamala, K.H. Richman, M.J. Brauer, B. Irvine, M.S. Urdea, P. Tekamp-Olson G. Kuo, Q.-L. Choo and M. Houghton, Proc. Natl. Acad. Sci. USA (1991) 88:1711 1715.

97. "Hepatitis C virus replication in 'autoimmune' chronic hepatitis", S. Magrin, A. Craxi, C Fabiano, G. Fiorentino, P. Almasio, U. Palazzo, G. Pinzello, G. Provenzano, L. Pagliaro Q.-L. Choo, G. Kuo, A. Polito, J. Han and M. Houghton, J. Hepatology (1991) 13:364 367.

98. "Use of a signature nucleotide sequence of Hepatitis C virus for detection of viral RN in human serum and plasma", T.-A. Cha, J. Kolberg, B. Irvine, M. Stempien, E. Beall, M Yano, Q.-L. Choo, M. Houghton, G. Kuo, J. H. Han and M. S. Urdea, J. Clin Microbiology (1991) 29:2528-2534.

99. "Molecular virology of HCV", M. Houghton, In Report on the Proceedings, Secon International Symposium on HCV, Los Angeles, Bradley, D.W. ed., 1991, Advance Therapeutics Communications, Secaucus, New Jersey, pp. 2-3.

100. "Replication and antigenic expression of HCV antiviral antibody response and live pathology in acute and chronic HCV infection", K. Krawczynski, M. Beach, L. Mimms E. Meeks, D. Vallari, S. Taskar, G. Kuo, M. Houghton and D. Bradley, Hepatolog (1991) 14:78.

101. "Molecular biology of the hepatitis C viruses: Implications for diagnosis, developmen and control of viral disease", M. Houghton, A. Weiner, J. Han, G. Kuo and Q.-L. Choo Hepatology (1991) 14:381-388.

102. "Hepatitis C antibody in patients with chronic liver disease and hepatocellular carci noma", M. Colombo, M. G. Rumi, M. F. Donato, M. A. Tommasini, E. Del Ninno, G Ronchi, G. Kuo and M. Houghton, Dig. Dis. Sci. (1991) 36:1130-1133.

103. "Expression and characterization of HCV structural proteins using in-vitro translation and recombinant vaccinia viruses", K. Thudium, R. Spaete, K. Berger, Q.-L. Choo, M Houghton and R. Ralston, J. Cell. Biochem. (1991) 15:92.

104 "Hepatitis C virus antibodies in acute icteric and chronic non-A, non-B hepatitis", J.C McHutchison, G. Kuo, M. Houghton, Q.-L. Choo and A.G. Redeker, Gastroenterolog (1991) 101:1117-1119.

105. "Hepatitis C virus markers for monitoring interferon therapy in chronic hepatitis C", G Saracco, M. Baldi, P. L. Calvo, P. Manzini, M. Abate, E. Chiaberge, M.R. Brunetto, M Rizzetto, D. Chien, G. Kuo, M. Houghton and F. Bonino, Hepatology (1991) 14:75A.

106. "Analysis of genomic variability of hepatitis C virus", H.T. Cuypers, I.N. Winkel, C.L. van der Poel, H.W. Reesink, P.N. Lelie, M. Houghton, and A. Weiner, J. Hepatol. (1991 13:S15-19.

107. "Rabbit-derived anti-HD antibodies for HDAg immunoblotting", F. Rosina, A. Fabiano A. Garripoli, A. Smedile, A. Mattalia, M.R. Eckart, M. Houghton, and F. Bonino, J Hepatol. (Netherlands) (1991) 13(S4):130-133.

108. "Sequence variation in hepatitis C viral isolates", A.J. Weiner, C. Christopherson, J.E Hall, F. Bonino, G. Saracco, K. Crawford, C.D. Marion, K.A. Crawford, S Venkatakrishna, T. Miyamura, J. McHutchinson, T. Cuypers, and M. Houghto Hepatology (1991) 13:S6-14.

109. "Hepatitis C virus antigen in hepatocytes: Immunomorphologic detection and identi cation", K. Krawczynski, M.J. Beach, D.W. Bradley, G. Kuo, A.M. di Bisceglie, M Houghton, G.R. Reyes, J.P. Kim, Q.-L. Choo, and M.J. Alter, Gastroenterology (199 103:622-629.

110. "Disappearance of hepatitis C virus RNA in plasma during interferon alpha-2B trea ment in hemophilia patients", D. Bresters, E.P. Mauser-Bunschoten, H.T. Cuypers P.N. Lelie, J.H. Han, P.L. Jansen, M. Houghton, and H.W. Reesink, Scand. J Gastroenterol. (1992) 27:166-168.

111. "Enhanced sensitivity of a second generation ELISA for antibody to hepatitis C virus D. Bresters, H.T. Cuypers, H.W. Reesink, W.P. Schaasberg, C.L. van der Poel, E.F Mauser-Bunschoten, M. Houghton, Q.-L. Choo, G. Kuo, R. Lesniewski, et al., Vo Sang (1992) 62:213-217.

112. "Early antihepatitis C virus response with second-generation C200/C22 ELISA", C.L van der Poel, D. Bresters, H.W. Reesink, A.A. Plaisier, W. Schaasberg, A. Leentvaa Kuypers, Q.-L. Choo, S. Quan, A. Polito, and M. Houghton, Vox Sang (1992) 62:208 212.

113. "Hepatitis Viruses", L.R. Overby and M. Houghton. In "Laboratory diagnosis of vir infections", (ed.: E.H. Lennette) (1992) Marcel Dekker, New York, pp. 403-441.

114. "Characterization of the hepatitis C virus E2/NS1 gene product expressed in mam malian cells", R.R. Spaete, D'A. Alexander, M.E. Rugroden, Q.-L. Choo, K. Berger, K Crawford, C. Kuo, S. Leng, C. Lee, R. Ralston, K. Thudium, J.W. Tung, G. Kuo and M Houghton, Virol. (1992) 189:1-12.

115. "Evidence for immune selection of hepatitis C virus (HCV) putative envelope glyco protein variants: Potential role in chronic HCV infections", A.J. Weiner, H.M. Geyser C. Christopherson, J.E. Hall, T.J. Mason, G. Saracco, F. Bonino, K. Crawford, C.D Marion, K.A. Crawford, M. Brunetto, P.J. Barr, Tatsuo Miyamura, J. McHutchinson and M.Houghton, Proc. Natl. Acad. Sci. USA (1992) 89:3468-3472.

116. "Heterogeneity of the HCV Genome: Importance for Control of the Disease", M Houghton, In: Hepatitis C Virus: Scientific and Clinical Status, Deinhardt, F., Bradle D.W., Houghton, M. eds., 1992, Advanced Therapeutics Communications, Secaucus New Jersey, pp. 8-9.

117. "Prevalence of antibodies to hepatitis C virus among patients with cryptogenic chron hepatitis and cirrhosis", L.J. Jeffers, F. Hasan, M. De Medina, R. Reddy, T. Parker, M Silva, L. Mendez, E.R. Schiff, M. Manns, and M. Houghton, Hepatology (1992) 15:187 190.

118. "The hypervariable amino terminus of the hepatitis C virus E2/NS1 protein appears t be under immune selection", A.J. Weiner, C. Christopherson, J.E. Hall, K. Crawford C.D. Marion, K.A. Crawford, P.J. Barr, K. Richman, G. Kuo and M. Houghton, In Vaccines 92, Brown, F., Chanock, R.M., Ginsberg, H.S., Lerner, R.A. eds., Cold Sprin Harbor Laboratory 1992, New York, pp 303-308.

119. "Diagnosis of hepatitis C virus (HCV) infection using an immunodominant chimeri polyprotein to capture circulating antibodies: Re-evaluation of the role of HCV in live disease", D.Y. Chien, Q.-L. Choo, A. Tabrizi, C. Kuo, J. McFarland, K. Berger, C. Lee J.R. Shuster, Tuan Nguyen, D.L. Moyer, M. Tong, S. Furuta, M. Omata, H. Alter, E Schiff, L. Jeffers, M. Houghton, and G. Kuo, Proc. Natl. Acad. Sci. USA (1992 89:10011-10015.

120. "5' end-dependent translation initiation of hepatitis C viral RNA and the presence : putative positive and negative translational control elements within the 5' untranslate region", B.J. Yoo, R.R. Spaete, A.P. Geballe, M. Selby, M. Houghton, and J.H. Han Virology (1992) 191:889-899.

121. "Identification of the major, parenteral non-A, non-B hepatitis agent (hepatitis C virus using a recombinant cDNA approach", Q.-L. Choo, G. Kuo, A. Weiner, K.-S. Wang, J. Overby, D. Bradley, and M. Houghton, Sem. Liver Dis. (1992) 12:279-288.

122. "Intrahepatic cytotoxic T lymphocytes specific for hepatitis C virus in persons wit chronic hepatitis", M.J. Koziel, D. Dudley, J.T. Wong, J. Dienstag, M. Houghton, F Ralston, and B. Walker, J. immunol. (1992) 149:3339-3344.

123. "Storage conditions of blood samples and primer selection affect the yield of cDN polymerase chain reaction products of hepatitis C virus", H.T. Cuypers, D. Bresters I.N. Winkel, H.W. Reesink A.J. Weiner, M. Houghton, C.L. van der Poel, and P.N. Lelie J. Clin. Microbiol. (1992) 30:3220-3224.

124. "PCR: Application to hepatitis C virus (HCV) research and diagnostics", A.J. Weiner, S Venkatakrishna, J.E. Hall, M. Houghton, and J. Han, In: Frontiers in Virology, Becke Y., Darai, G. eds., Springer Verlag, New York 1992, pp 86-100.

125. "T cell response to recombinant proteins of hepatitis C virus in blood and liver c patients with different clinical courses of infection", P. Botarelli, M.R. Brunetto, A. Weiner, M.A. Minutello, D. Unutmaz, P. Calvo, F. Bonino, M. Houghton, and S Abrignani, Gastroenterology (1993) 104:580-587.

126. "Early antihepatitis-C virus response with second generation C200/C22 ELISA", C.L Van der Poel, D. Bresters, H.W. Reesink, A.A.D. Praisler, W. Schaasberg, A. Leentvaar Kuypers, Q.-L. Choo, S. Quan, A. Polito, M. Houghton, G. Kuo, P.N. Lelie, and H.T.M Cuypers, Vox Sanguinis (1992) 62:208-212.

127. "Group specific sequences and conserved secondary structures at the 3' end of HCV genome and its implication for viral replication", J.H. Han and M. Houghton, Nucl. Acid. Res. (England) (1992) 20:3520.

128. "A unique, predominant hepatitis C virus variant found in an infant born to a mothe with multiple variants", A.J. Weiner, M.M. Thaler, K. Crawford, K. Ching, J. Kansopon D.Y. Chien, J.E. Hall, F. Hu, and M. Houghton, J. Virol. (1993) 67:4365-4368.

129. "Expression, identification and subcellular localization of the proteins encoded by the hepatitis C viral genome", M.J. Selby, Q.-L. Choo, K. Berger, G. Kuo, E. Glazer, M Eckart, C. Lee, D. Chien, C. Kuo, and M. Houghton, J. Gen. Virol. (1993) 74:1103 1113.

130. "Persistence of HCV despite antibodies to both putative envelope glycoproteins", D.Y Chien, Q.-L. Choo, R. Ralston, R. Spaete, M. Tong, M. Houghton, and G. Kuo, Lance (1993) 342:933.

131. "Use of recombinant HCV antigen in the serodiagnosis of hepatitis C virus infection Significant improvement in HCV antibody detection as compared with the first genera tion HCV C100-3 ELISA and the synthetic peptide EIA tests", D.Y. Chien, Q.-L. Choo A. Tabrizi, C. Kuo, J. McFarland, K. Berger, C. Lee, J.R. Shuster, T. Nguyen, D.L Moyer, M. Tong, S. Furuta, M. Omata, C.T. Fong, G. Tegtmeier, H. Alter, E. Schiff, L Jeffers, M. Houghton, and G. Kuo, J. Gastroenterology and Hepatology (1993) 8:S33 39.

132. "The hepatitis C virus encodes a serine protease involved in processing of the putativ nonstructural proteins from the viral polyprotein precursor", M.R. Eckart, M. Selby, F Masiarz, C. Lee, K. Berger, K. Crawford, C. Kuo, G. Kuo, and M. Houghton, Biochem and Biophys. Res. Comm. (1993) 192:399-406.

133. "Antibody response to core, envelope and nonstructural hepatitis C virus antigen Comparison of immunocompetent and immunosuppressed patients", A.S.F. Lok, J Chien, Q.-L. Choo, T.-M. Chan, E.K.W. Chiu, I.K.P. Cheng, M. Houghton, and G. Ku Hepatology (1993) 18:497-502.

134. "Characterization of hepatitis C virus envelope glycoprotein complexes expressed i recombinant vaccinia viruses", R. Ralston, K. Thudium, K. Berger, C. Kuo, B. Gervas J. Hall, M. Selby, G. Kuo, M. Houghton, and Q.-L. Choo, J. Virol. (1993) 67:6753-6761

135. "Long-term follow-up of patients with chronic hepatitis C treated with different doses interferon-α_{2b}", G. Saracco, F. Rosina, M.L. Abate, L. Chiandussi, V. Gallo, E. Cerut A. Di Napoli, A. Solinas, A. DePlano, A. Tocco, P. Cossu, D. Chien, G. Kuo, A. Polit A.J. Weiner, M. Houghton, G. Verme, F. Bonino, and M. Rizzetto, Hepatology (199 18:1300-1305.

136. "Hepatitis C virus (HCV)-specific cytotoxic T lymphocytes recognized epitopes in th core and envelope proteins of HCV", M.J. Koziel, D. Dudley, N. Afdhal, Q.-L. Choo, M Houghton, R. Ralston, and B.D. Walker, J. Virol. (1993) 67:7522-7532.

137. "Hepatitis C virus-specific CTL responses in the liver of chimpanzees with acute an chronic hepatitis C", A.L. Erickson, M. Houghton, Q.-L. Choo, A.J. Weiner, R. Ralsto E. Muchmore, and C.M. Walker, J. Immunol. (1993) 151:4189-4199.

138. "Compartmentalization of T lymphocytes to the site of disease: intrahepatic CD4+ cells specific for the protein NS4 of hepatitis C virus in patients with chronic hepatit C", M.A. Minutello, P. Pileri, D. Unutmaz, S. Censini, G. Kuo, M. Houghton, M. Brunetto, F. Bonino, and S. Abrignani, J. Exp. Med. (1993) 178:17-25.

139. "Long term treatment of chronic hepatitis C with interferon alfa-2b: disappearance HCV-RNA in a pilot study of eight haemophilia patients", D. Bresters, E.P. Mause Sunschoten, H.T. Cuypers, J.H. Han, P.L. Jansen, R.A. Chamuleau, M. Houghto and H.W. Reesink, Gut (England) (1993) 34:124-125.

140. "Rabbit-derived anti-HD antibodies for HDAg immunoblotting", F. Rosina, A. Fabian, E. Maran, R. Cozzolongo, A. Smedile, G. Mazzucco, A. Garripoli, C. Costa, M.R. Eckar and M. Houghton, Proc. Clin. Biol. Res. (1993) 382:189-191.

141. "Vaccination of chimpanzees against infection by the hepatitis C virus", Q.-L. Choo, G Kuo, R. Ralston, A. Weiner, D. Chien, G. Van Nest, J. Han, K. Berger, K. Thudium, C Kuo, J. Kansopon, J. McFarland, A. Tabrizi, K. Ching, B. Moss, L.B. Cummins, M Houghton, and E. Muchmore, Proc. Natl. Acad. Sci. USA (1994) 91:1294-1298.

142. "A proposed system for the nomenclature of hepatitis C viral genotypes", I Simmonds, A. Alfredo, H.J. Alter, F. Bonino, D.W. Bradley, C. Brechot, J.T. Brouwer, S W. Chan, K. Chayama, D.-S. Chen, Q.-L. Choo, M. Colombo, H.T.M. Cuypers, T. Dat G.M. Dusheiko, J.I. Esteban, O.Fay, S.J. Hadziyannis, J. Han, A. Hatzakis, E.C Holmes, H. Hotta, M. Houghton, B. Irvine, M. Kohara, J.A. Kolberg, G. Kuo, J.Y.N. La P.N. Lelie, G. Maertens, F. McOmish, T. Miyamura, M. Mizokami, A. Nomoto, A.M Prince, H.W. Reesink, C. Rice, M. Roggendorf, S.W. Schaim, K. Shimotohno, J Stuyver, C. Trépo, A. Weiner, P.L. Yap, and M.S Urdea, Hepatology (1994) in press.

143. "Hepatitis C Virus: Structure, protein products and processing of the polyprote precursor", M. Houghton, M. Selby, A. Weiner, and Q.-L. Choo, in Reesink, H.W. (ec Hepatitis C Virus. Curr. Stud. Hematol. Blood Trans. Karger, Basel, Switzerland (199 61:1-11.

144. "The Hepatitis C Virus: Genetic organization, persistence, and vaccine strategies", M Houghton, Q.-L. Choo, G. Kuo, R. Ralston, M. Selby, A. Weiner, D. Chien, J. Han, C Walker, S. Abrignani, M. Koziel, B. Walker, L. Cummins, and E. Muchmore, In Nishiok K., Suzuki, H., Mishiro, S., Oda, T. (eds.) Viral Hepatitis and Liver Disease. Springe Verlag, Tokyo (1994) pp. 33-37.

145. "HCV-positive, HIV-1-negative mothers transmit HCV", A.J. Weiner, M.M. Thaler, H Crawford, J. Kansopon, K. Ching, J.E. Hall, F. Hu, D. Chien, and M. Houghton, I Nishioka, K., Suzuki, H., Mishiro, S., Oda, T. (eds.) Viral Hepatitis and Liver Disease Springer-Verlag, Tokyo (1994) pp. 463-467.

146. "Complex processing and protein: Protein interactions in the E2:NS2 region of HCV M.J. Selby, E. Glazer, F. Masiarz, and M. Houghton, Virology (1994) 204:114-122.

147. "Peptide immunogen mimicry of putative E1 glycoprotein-specific epitopes in hepatiti C virus", R. Ray, A. Khanna, L.M. Lagging, K. Meyer, Q.-L. Choo, R. Ralston, M Houghton, and P.R. Becherer, J. Virol. (1994) 68:4420-4426.

148. "Hepatitis C virus markers in patients with long-term biochemical and histologic remission of chronic hepatitis", G. Saracco, M.L. Abate, M. Baldi, P.L. Calvo, F Manzini, M.R. Brunetto, F. Oliveri, G. Kuo, D. Chien, and M. Houghton, et al., Live (1994) 14:65-70.

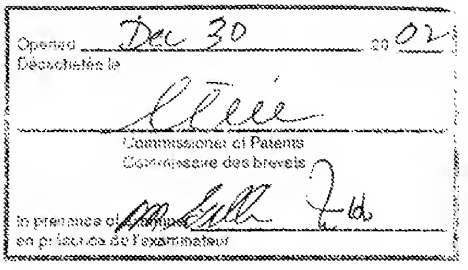
149. "Transfection of a differentiated human hepatoma cell line (Huh7) with in vitro transcribed hepatitis C virus (HCV) RNA and establishment of a long-term cultur persistently infected with HCV", B.J. Yoo, M.J. Selby, J. Choe, B.S. Suh, S.H. Choi, J.S Joh, G.J. Nuovo, H.-S. Lee, M. Houghton, and J.H. Han, J. Virol. (1995) 69:32-38.

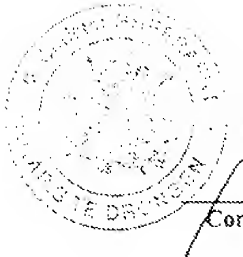
150. "Cytotoxic T lymphocyte response to hepatitis C virus—Derived peptides containing the HLA A2.1 binding motif", A. Cerny, J.G. McHutchison, C. Pasquinelli, M.E. Brown M.A. Brothers, B. Grabscheid, P. Fowler, M. Houghton, and F.V. Chisari, J. Clin. Invest (1995) 95:521-530.

151. "HLA class I-restricted cytotoxic T lymphocytes specific for hepatitis C virus Identification of multiple epitopes and characterization of patterns of cytokine release" M.J. Koziel, D. Dudley, N. Afdhal, A. Grakoui, C. Rice, Q.-L. Choo, M. Houghton, and B.D. Walker, (1995) submitted for publication

152. "Persistent hepatitis C virus infection in a chimpanzee is associated with emergence o a cytotoxic T lymphocyte escape variant", A. Weiner, A.L. Erickson, J. Kansopon K. Crawford, E. Muchmore, A.L. Hughes, M. Houghton, and C.M. Walker, Proc. Natl Acad. Sci. USA (1995) 92:2755-2759.

153. "Induction in vitro of a primary human antiviral cytotoxic T cell response," A. Cerny, P Fowler, M.A. Brothers, M. Houghton, H.J. Schlicht, and F.V. Chisari, Eur. J. Immunol (1995) 25:627-630.





This is EXHIBIT FIERS-31
to
the Affidavit of Walter C. Fiers
sworn before me
this 19 th day of November, 2001

Commissioner for Oath or Notary Public

Opposition to European Patent No. 0 041 313 of Biogen, Inc.
Schering Aktiengesellschaft
Appeal File No. T0207/94-334

AFFIDAVIT OF DR. RIK DERYNCK

I, RIK DERYNCK, declare that:

1. I am a citizen of Belgium, and reside at 2 Clark Dr., Apt. 320, San Mateo, CA 94401.

2. I am a Professor in the Department of Growth and Development, the Department of Anatomy, and the Programs of Cell Biology and Developmental Biology, at the University of California, San Francisco, California, U.S.A.

My scientific expertise in the field of Molecular Biology is established by my curriculum vitae, which is attached.

3. In the years 1978-1980, several research groups were engaged in an intense, competitive effort to clone and express the gene for human fibroblast interferon-β (IFN-β). I was a member of one such group, centered at the Laboratory of Molecular Biology, State University of Ghent, in Ghent, Belgium, in the laboratory of Dr. Walter Fiers. This research was sponsored in part by Biogen N.V., now Biogen, Inc. My task was to clone the IFN-β cDNA, and then to insert it in an expression vector to express IFN-β in *E. coli*.

4. After the announcement by Taniguchi in late February 1980 that he had isolated the full size coding sequence of IFN-β, we continued and even increased our efforts to express recombinant IFN-β.

5. The primary goal of this project in our laboratory in expressing the IFN-β cDNA was not to maximize the yield or to achieve commercially or pharmaceutically useful levels of IFN-β in a pure state. These were issues to be later solved by Biogen, or to be the subject of a future project in our laboratory. Rather, our goal was to achieve a finite level of expression of IFN-β, however low, in order to prove the feasibility of the commercial production of the recombinant protein, as well as to be the first group to publish on the recombinant synthesis of IFN-β. We pursued this goal with the methodology available to us at that time, and no new technology was required to achieve the expression of IFN-β, which we accomplished about two months after obtaining possession of the full length cDNA encoding it.

Dr. Rik Derynck

Date 9/26/96

Case Number: T 0207/94 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 8 April 1997

Appellant: (Opponent)
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Decision under appeal: Decision of the Opposition Division of the European Patent Office posted 21 January 1994 rejecting the opposition filed against European patent No. 0 41 313 pursuant to Article 102(2) EPC.

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: F. L. Davison-Brunel
W. Moser

Summary of facts and submissions

I. European patent No. 0 041 313 (application No. 81 301 414.9) relating to "DNA sequences, recombinant DNA molecules and processes for producing human fibroblast interferon" and claiming priority from GB 8011306 of 3 April 1980 and GB 8018701 of 6 June 1980 was granted for eleven contracting states with 18 claims (15 claims for AT).

Claims 1, 17 and 18 for all contracting states other than AT read:

"1. A recombinant DNA molecule capable of inducing the expression in a unicellular host of a polypeptide displaying the immunological or biological activity of human beta-interferon, said molecule comprising a DNA sequence selected from:

(a) the DNA inserts of G-pPLa-HFIF-67-12 (*Hinc*II-*Sau*3AI), G-pPLa-HFIF-67-12 Δ19 (*Hinc*II-*Sau*3AI), and G-pPLc-HFIF-67-8 (*Hinc*II-*Sau*3AI) carried by the microorganisms identified by accession numbers DSM 1851-1954, respectively,

(b) DNA sequences which hybridize to any of the foregoing DNA inserts, and

(c) DNA sequences which are degenerate as a result of the genetic code to the DNA inserts and sequences defined in (a) and (b) and which code on expression for a polypeptide having the same amino acid sequence,

said DNA sequence being operatively linked to an expression control sequence in said recombinant DNA molecule."

"17. A composition for treating human viruses, for treating human cancers or tumors, or for immunomodulation which comprises as a sole IFN-beta a polypeptide produced according to any one of claims 12 to 16."

"18. The use as a sole IFN-beta of a polypeptide produced according to any one of the claims 12 to 16, for the manufacture of a composition for treating human viruses, for treating human cancers or tumors, or for immunomodulation."

Claims 2 to 7 specified further embodiments of the recombinant DNA molecule of claim 1. Claims 8 to 10 were directed to unicellular hosts transformed with the claimed recombinant DNA molecules. Claim 11 was directed to a method for making these unicellular hosts. Claims 12 to 16 were addressed to methods for producing the polypeptide encoded by the recombinant DNA molecule according to anyone of claims 1 to 7.

The corresponding claims were granted for AT.

II. A notice of opposition was filed against the European patent. Revocation of the patent was requested on the grounds of Article 100(a) EPC (lack of novelty and inventive step) and Article 100(b) EPC (insufficiency of disclosure).

III. In the course of the procedure, one hundred and forty-three documents were filed. Of these, the following were in particular relied upon by the parties and are referred to in the present decision:

(1) : EP-B-0 028 033,

(2) : Taniguchi et al., Gene 10, pages 11 to 15, 1980,

(3) : Nagata et al, Nature 284, pages 316 to 320, 1980,

(7) : Taniguchi et al., Proc. Japan. Acad. Ser.B, pages 464 to 469, 1979,

(9) : Itakura et al., Science 198, pages 1056 to 1063, 1977,

(10) : Villa-Komaroff et al.,Proc. Natl. Acad. Sci.USA 75, pages 3727 to 3731, 1978,

(12) : Martial et al., Science 205, pages 602 to 606, 1979,

(14) : Taniguchi et al., Proc. Natl. Acad. Sci. USA 77, pages 5230 to 5233, 1980,

(16) : Houghton, M., Nature 285, page 536, 1980,

(17) : Stüber and Bujard, Proc. Natl. Acad. Sci. USA 78, pages 167 to 171, 1981,

(19) : Table: Eucaryotic genes cloned and expressed prior to June 6, 1980, received on 2 November 1993,

(21) : Goeddel et al., Nucl. Acids Res. 8, pages 4057 to 4074, 1980,

(22) : Goeddel et al., Nature 281, pages 544 to 548, 1979,

(23) : Roberts et al., Proc. Natl. Acad. Sci. USA 76, pages 5596 to 5600, 1979,

(29) : Taniguchi and Weissmann, J. Mol. Biol. 118, pages 533 to 565, 1978,

(34) : Mercereau-Puijalon et al., Nature 275, pages 505 to 510, 1978,

(41) : Holmgren, A., The Journal of Biochemistry 254, No.18, pages 9113 to 9119, 1979,

(53) : Guarente et al., Cell 20, pages 543 to 553, 1980

(62) : Derynck et al., Nature 287, pages 193 to 197, 1980,

(63) : Havell et al., Proc. Natl. Acad. Sci. USA, 72, pages 2185 to 2187, 1975,

(66) : Vilcek et al., Ann. N. Y. Acad. Sci. 284, pages 703 to 710, 1977,

(77) : Weissenbach et al., Eur. J. Biochem. 98, pages 1 to 8, 1979,

(81) : Sulkowski et al., Ann. N. Y. Acad. Sci. 350, pages 339 to 346, 1980,

(82) : Jankowski et al., Biochemistry 15, pages 5182 to 5187, 1976,

(89) : EP-B-0 034 306

(101) : Shepard et al., Nature 294, pages 563 to 565, 1981,

(122) : Patentee's experimental report received on 10 March 1997,

(132) : Declaration of Dr. M. A. Innis of 6 March 1997.

IV. The opposition division issued a decision whereby the opposition was rejected under Article 102(2) EPC and the patent was maintained as granted.

V. The opposition division considered that sufficient information was given in the patent specification on how to test the interferon (IFN) biological and immunological activities and on how to isolate and identify the variants of claims 1(b) and (c) for the requirements of Article 83 EPC to be fulfilled.

Priority (Articles 87 to 89 EPC) was seen to be valid from 6 June 1980 for claim 1 and its dependent claims, and from 1 April 1981 for claims 2 and 3 and their dependent claims.

Novelty (Article 54 EPC) was acknowledged over document (1) or (2) because these documents did not provide convincing evidence that the plasmid they disclosed could have expressed IFN-beta from any of the pBR322 promoters. The experimental data submitted by the opponent did not credibly show that the anti-viral activity seen in hosts containing said plasmid was due to the expression of the human IFN-beta gene.

With regard to inventive step, the closest prior art was identified as document (2) and the technical problem to be solved was defined as the recombinant production of a polypeptide displaying the immunological or biological activity of human IFN-beta.

The opposition division found that human IFN-beta could not have been expressed in a straightforward manner by the then existing methods of expression. Furthermore, in view of the physical and chemical differences between IFN-beta and the mammalian proteins which had already been produced in recombinant form, successful expression could not have been predicted. Inventive step was, thus, acknowledged.

VI. The appellant (opponent) lodged an appeal against the decision of the opposition division, at the same time paying the appeal fee. The statement of grounds of appeal was submitted.

VII. The respondent (patentee) filed a response to the grounds of appeal, followed by further submissions by both parties.

VIII. The board issued a communication pursuant to Article 11(2) of the Rules of Procedure of the Boards of Appeal, setting out the board's provisional position.

IX. The board's communication was followed by further submissions from both parties. The respondent filed one new main request which differed from the granted set of claims in that claims 2, 3, 7 and 10 were deleted and the other claims renumbered accordingly. Claims 1, 13 and 14 thus remained the same as granted claims 1, 17 and 18 respectively (see point I supra).

X. Oral proceedings were held on 8 and 9 April 1997. Two new auxiliary requests were submitted. New auxiliary request I was withdrawn at a later stage in the oral proceedings. New auxiliary request II differed from the main request in that the feature "displaying the immunological or biological activity of human beta-interferon" was replaced by the feature "displaying the biological activity of human beta-interferon" in all of the claims containing it. In particular, claim 1 read:

"1. A recombinant DNA molecule capable of inducing the expression in a unicellular host of a polypeptide displaying the biological activity of human beta-interferon, said molecule comprising a DNA sequence selected from:

(a) the DNA inserts of G-pPLa-HFIF-67-12 (*HincII-Sau3AI*), G-pPLa-HFIF-67-12 19 (*HincII-Sau3AI*), and G-pPLc-HFIF-67-8 (*HincII-Sau3AI*) carried by the microorganisms identified by accession numbers DSM 1851-1954, respectively,

(b) DNA sequences which hybridize to any of the foregoing DNA inserts, and

(c) DNA sequences which are degenerate as a result of the genetic code to the DNA inserts and sequences defined in (a) and (b) and which code on expression for a polypeptide having the same amino acid sequence,

said DNA sequence being operatively linked to an expression control sequence in said recombinant DNA molecule."

XI. The submissions in writing and during oral proceedings by the appellant were essentially as follows:

Priority:

For a priority application to establish valid priority rights, it was necessary that it be enabling and disclosed all of the essential features of the claimed subject-matter. According to the case law of the EPO (T 409/91, OJ EPO 1994,653, T 435/91, OJ EPO 1995,188) enablement could only be acknowledged if the envisaged result could be achieved without undue burden within the whole ambit of the claim.

The second priority document formally disclosed the three specific plasmids of claim 1(a) as well as the variants of claim 1(b) and (c), but it was not enabling. One of the specific plasmids of claim 1(a) was later shown not to produce a polypeptide with beta-IFN biological activity (document (62)), whereas the other two directed the synthesis of fusion proteins which were bound to have properties different from those of mature beta-IFN. The step of proteolytic cleavage which would be necessary to make them into mature recombinant interferon was not mentioned. Furthermore, document (16) indicated that mature recombinant beta-IFN did not have the same molecular weight as natural mature beta-IFN. Finally, the appellant had submitted an affidavit (document (132)) which, in the appellant's view, provided proof that none of the plasmids had the required properties.

Isolating the DNA variants of claim 1(b) and (c) and testing whether they expressed muteins with beta-IFN immunological or biological properties amounted to an undue burden of experimentation. There could be no certainty that any of the muteins would be active as no

natural active mutein of beta-IFN was known. Document (101) disclosed a mutein without beta-IFN activity.

The second priority application did not disclose the production of beta-IFN in such an amount and of such a quality that it could be made into the pharmaceutical preparation of claims 13 and 14 (granted claims 17 and 18).

Because of lack of enablement, the second priority application could not serve to establish valid priority rights.

Sufficiency of disclosure

The subject-matter of claims 1, 13 and 14 of all requests was no more substantiated in the specification of the patent in suit than in the second priority document. The requirements of Article 83 EPC were not fulfilled.

Novelty

Document (1) or (2) disclosed the plasmid TpIF319-13 in which the beta-IFN cDNA had been inserted into the EcoRI site of pBR322. At this position, the cDNA could be transcribed from the pBR322 P4 promoter. Experimental evidence had been provided by the appellant of antiviral activity in a lysate of E.coli cells containing said plasmid.

The same process which led to the isolation of TpIF319-13 would equally result in the cDNA being inserted in the opposite orientation in the EcoRI site. In this case, it could be transcribed from the pBR322 P1 promoter. Furthermore, it was stated in document (1), page 11, lines 10 to 15, that the transformation of

beta-IFN cDNA to other expression plasmids would enable a host such as E.coli to produce beta-IFN. Document (1) or (2) was, thus, detrimental to the novelty of claim 1 under Article 54(2) EPC.

Document (89) was also detrimental to novelty under Article 54(3) EPC. This latter document disclosed a process for the isolation of beta-IFN with two alternative screening methods for the recombinant clones. This process would necessarily and inevitably enable the isolation of the clone expressing the beta-IFN cDNA, as an analogous process had previously permitted the recovery of 184 alpha-IFN cDNA recombinant clones out of 5 000 transformants (document (3)).

The subject-matter of claims 13 and 14 also lacked novelty over documents (66) and (77) which disclosed pure natural beta-IFN, as the claimed pharmaceutical preparations containing recombinant beta-IFN could not be distinguished from those containing pure natural beta-IFN.

Inventive step:

The closest prior art was document (2) which disclosed the cloning and nucleotide sequence of beta-IFN cDNA. The problem to be solved could be defined as expressing beta-IFN in detectable amounts from this DNA sequence. The solution, which consisted in making a construct where the beta-IFN cDNA was linked to a promoter known to be active in the chosen host cells, resulted in very low amounts of beta-IFN being made.

Document (22) or (23) disclosed expression systems which were suitable for the expression of beta-IFN as shown in post-published document (21) or (14). Document (3) described the cloning and expression of

alpha-IFN cDNA. For a person skilled in the art, it would have been obvious to try combining the teachings of document (2) with those of any of these documents to solve the above-stated problem.

A reasonable expectation of success did exist since it was already known that beta-IFN mRNA was stable in heterologous host cells, that full glycosilation was not necessary for the protein to be active and that the protein properly folded after denaturation and renaturation.

The properties of beta-IFN were not so different from those of many eucaryotic proteins which had already been expressed in recombinant form (document (19)) that difficulties may have been foreseen. Beta-IFN resembled alpha-IFN in terms of its hydrophobicity and of the number of Cys residues it contained. The presence of the rare Ile codon AUA in its coding sequence was not important since other mammalian proteins with the same codon in their coding sequence had already been expressed in recombinant form (document (22)). There was no conceivable reason why the proximity of two AUG codons at the 5' end of the gene would interfere with expression.

XII. The respondent's answer was essentially as follows:

Priority; sufficiency of disclosure:

No essential features were lacking in the second priority application to make the invention work, and the invention could be carried out from the instructions given therein without undue burden over the whole ambit of the claim.

All of the plasmids disclosed in the second priority application had beta-IFN biological activity as could be seen from pages 71 and 72 of the application.

Document (62) did not report that one of the claimed plasmids had no biological activity but rather that variable results were obtained. The experimental conditions used by the appellant to test whether the plasmids directed the synthesis of polypeptides with the properties of beta-IFN were too far away from the conditions used in the patent in suit to prove that the activities of these polypeptides could not repeatedly be obtained. It was clear from the fact that the recombinantly produced beta-IFN was active that proteolytic cleavage spontaneously occurred. Thus, this step did not need to be specifically mentioned. It was not relevant to enablement that different values of molecular weight had previously been obtained, as the molecular weight depended on which form of beta-IFN had been tested.

The second priority application (page 80) provided useful information on how to modify the already known beta-IFN DNA sequence, and testing for beta-IFN activity only required a very simple assay. Thus, the subject-matter of claim 1(b) and (c) could easily be obtained. It was EPO practice to grant claims to hybridising DNA sequences to a known DNA sequence.

Claims 13 and 14 of all requests also enjoyed priority rights from the second priority application because said application contained clear evidence that beta-IFN had been synthesised from the disclosed plasmids and, starting from this result, it was possible to formulate a pharmaceutically suitable preparation.

For all of these reasons, the second priority application enabled the invention. This conclusion also applied to the specification of the patent in suit which comprised the same information as the second priority application.

Novelty:

At their publication date, neither document (1) nor document (2) disclosed the fine molecular structure of the plasmid alleged to destroy novelty (TpIF319-13), even implicitly, since the P1 and P4 promoters had not yet been identified. In TpIF319-13, the beta IFN-cDNA was in the wrong orientation to be transcribed from P1. Furthermore, the mRNAs transcripts initiated from P4 would never reach the EcoRI site as they stopped some 200 nucleotides after P4 or in the **bla** gene. It was thus not possible that the plasmids would express beta-IFN, ie fall under the scope of the claim. The respondent had carried out activity tests on TpIF319-13 which had been negative (document (122)).

Arguing that document (1), page 11, lines 10 to 15, was novelty destroying to the subject-matter of claim 1 amounted to combining said document with any other document disclosing expression plasmids. Such a combination was clearly unallowable mosaic work in the context of assessing novelty.

The skilled person carrying out the process disclosed in document (89) would not necessarily obtain plasmids falling within the claim, because as many as 800 000 clones would have to be screened to have a 99% chance of obtaining one positive clone, which was an impossible experiment to carry out. The process according to document (3) which led to 184 positive clones out of 5 000 included one very important step which was missing in the process according to document (89).

Neither document (77) nor document (66) disclosed natural beta-IFNs in pure form. They could not destroy the novelty of the subject-matter of claims 13 and 14.

Inventive step:

Document (2) was the closest prior art. The technical problem to be solved was the recombinant production of a polypeptide displaying the immunological or biological activity of beta-IFN.

The argument that any of the combinations of document (2) (beta-IFN cDNA sequence) with document (22) or (23) (available expression systems) rendered the invention obvious because each of these combinations had successfully been used after the priority date to express beta-IFN could not be accepted because, in fact, none of the vectors of document (22) or (23) was ever used in constructs leading to beta-IFN expression. The authors of documents(22) and (23) chose different expression systems when they came to express recombinant beta-IFN (documents (21) and (14)).

In the same manner, the combination of document (2) with document (3) which disclosed the expression of alpha-interferon did not negate inventive step because of the many differences in the structure and properties of the alpha- and beta-IFNs.

There was no reasonable expectation of success that active beta-IFN could be retrieved from the recombinant hosts because of the properties of beta-IFN. Beta-IFN had a higher apparent hydrophobicity than alpha-IFN (document (81)), which would have caused doubts as to whether it would stick to cell membranes, thereby possibly causing host cell death or preventing its detectability. It contained three cystein residues and, thus, concerns would have existed that the wrong disulfide bridges would be formed (intra- or extramolecularly) in the reducing conditions found in the cytoplasm and in the absence of any glycosylation. The coding sequence of beta-IFN contained an unusual

codon for Ile, the effect of which on translation could not have been foreseen. The proximity of two ATGs at the 5' end of the coding sequence may also have disturbed translation.

For all of these reasons, inventive step must be acknowledged.

XIII. The appellant requested that the decision under appeal be set aside and that European patent No. 0 041 313 be revoked.

XIV. The respondent (patentee) requested that the decision under appeal be set aside and the patent be maintained on the basis of the following requests:

(a) main request: claims 1 to 14 filed on 10 March 1997

(b) auxiliary request: claims 1 to 14 for all designated contracting states, except AT, claims 1 to 11 for AT, submitted during oral proceedings as second auxiliary request.

Reasons for the decision

1. The appeal is admissible.

Main request

2. The main request differs from the granted set of claims in that claims 2, 3, 7 and 10 have been deleted. In claim 1, the deposited micro-organisms are defined by accession numbers 1851-1854 as in the originally filed application, page 94. None of these alterations amounts

to added subject-matter nor to an extension of the protection conferred. The requirements of Article 123(2) and (3) EPC are fulfilled.

3. The amendments to claim 1 do not render the claim unclear (Article 84 EPC).

Priority; Articles 87 to 89 EPC

4. There was agreement amongst the parties that the first priority application does not disclose the subject-matter of claims 1, 13 and 14. It remains to be decided whether the second priority date is valid.

5. The question at issue is whether the requirements of Article 87 EPC that the same invention is claimed in the priority application and the European patent application are fulfilled in the sense that the priority application discloses the invention in an enabling manner (See T 296/93, OJ EPO 1995, 627).

6. The second priority application (pages 55 to 60) discloses how to construct the three specific plasmids of claim 1(a), which are also identified by their deposit numbers (page 81). The examples show that they produce polypeptides with beta-IFN immunological or biological activity. Document (62) (to be taken as an expert document, page 195) confirms these results, although one of the plasmids synthesises only trace amounts of the protein.

7. The appellant also provided an affidavit where the three plasmids were tested for their properties (document (132)). Two of them were found to synthesise polypeptides with the immunological properties of beta-IFN. One of them produced a polypeptide with beta-IFN biological activity but to a small extent. The plasmid characterised in document (62) as a poor producer did

not express beta-IFN. The board notices that the methods used in document (132) (steps B and C) to extract and test the beta-IFN polypeptides are different in quite a number of respects from the methods described in the second priority application. It is thus not possible to infer from the half-way negative results of document (132) that the second priority application does not disclose the subject-matter of claim 1(a) in an enabling manner.

8. It has also been argued that, starting from the teachings of the second priority application, isolating and testing the DNA variants of claim 1(b) and (c) would amount to an undue burden of experimentation. The board however remarks that the sequence of beta-IFN cDNA was known from document (2). Chemical DNA synthesis or site-directed mutagenesis was available from the art (document (9) or (29)). Immunological and biological assays of beta-IFN were routinely carried out (documents (7) and (63)). Thus, in the board's judgment, while involving a non-negligeable amount of work, isolating the DNA variants of claim 1(b) and (c) would nonetheless have been quite feasible.

9. The concern voiced by the appellant that the person skilled in the art would not have considered it possible to isolate active beta-IFN protein variants because no such natural variants had ever been obtained does not seem to the board to be quite to the point since claim 1(b) and (c) does not relate to protein variants of beta-IFN but to DNA variants of beta-IFN cDNA. Any of the cDNA variants which differs from the cDNA of claim 1(a) by an alteration which does not induce a change in the protein sequence will necessarily lead to an active protein.

10. Claims 13 and 14 relate to pharmaceutical preparations containing recombinant beta-IFN. In case larger quantities of purer beta-IFN than were obtained in example C of the second priority application would be needed to make such preparations, the specification of this application provides on pages 77 to 80 information on how to produce pure interferon in large amounts. The board accepts that this information would permit said production.

11. The board concludes that the subject-matter of the claims of the main request is enabled by the second priority application. Consequently, priority has to be acknowledged from 6 June 1980.

Sufficiency of disclosure, Article 83 EPC

12. The specification of the patent in suit contains the same information as the second priority application with respect to the subject-matter of the claims of the main request. It is enabling for the same reasons as given under points 4 to 10 supra for said priority application.

Novelty, Article 54 EPC

13. Both documents (1) and (2) disclose a plasmid TpIF319-13 which carries the beta-IFN cDNA in the EcoRI site of pBR322 in such an orientation that it could theoretically be transcribed from the P4 promoter of pBR322 and which, if so in practice, could be novelty-destroying for the subject-matter of claim 1.

14. In the course of oral proceedings before the department of first instance, experiments were presented by the appellant to the effect that beta-IFN biological activity could be retrieved from host cells transformed with TpIF319-13. The opposition division found that

these experiments did not conclusively show that the detected antiviral activity could be unambiguously attributed to beta-IFN. The board is also unable to attach significance to these data since, according to Figure 1 of these experiments, the protection of the mammalian cells against the virus happens at dilutions of the TpIF319-13 bacterial extracts below 1:4 whereas, according to the third paragraph of the experimental results, mammalian cell growth is most inhibited at these dilutions.

15. Furthermore, document (17) (as an expert opinion) discloses that transcripts initiated at P4 are mostly 104 bp in length. A few mRNA molecules are of greater length but transoription stops into the **bla** gene before the EcoRI site. It thus does not seem possible that the beta-IFN cDNA would ever be transcribed from P4.

16. The data presented by the appellant do not provide convincing evidence that, contrary to what may theoretically be expected from the molecular structure of the plasmid (document (17)), said plasmid would direct the synthesis of beta-IFN.

17. It has further been argued that the general teaching in document (1) or (2) of the cloning of beta-IFN cDNA in the EcoRI site of pBR322 was novelty-destroying to the subject-matter of claim 1 since some recombinant clones would necessarily be obtained with the beta-IFN cDNA in such an orientation that it would be transcribed from the P1 promoter of pBR322. The board could agree that, on a statistical basis, one in two beta-IFN cDNA clones should carry the beta-IFN DNA insert in an orientation permitting its transcription from the P1 promoter. Yet evidence for the existence of such a clone is missing. Given the fact that document (1)(page 11) advises that the beta-IFN cDNA should be transferred from pBR322 to an expression vector in order to synthesise beta-IFN,

the board is not convinced that the general teaching in document (1) or (2) constitutes unambiguous evidence for a recombinant plasmid expressing beta-IFN from the P1 promoter of pBR322.

18. Accordingly, and in line with the case law of the EPO (see T 612/92 of 28 February 1996) that the teachings of a document belonging to the prior art must be unambiguous before they can be taken into account for assessing novelty, the board considers that neither document (1) nor document (2) are novelty destroying to the subject-matter of claim 1.

19. Document (89) was cited as novelty destroying under Article 54(3) EPC for the subject-matter of claim 1. It discloses a method for the isolation and screening of recombinant plasmids expressing beta-IFN cDNA or parts thereof. Document (89) does not provide any evidence that this method has ever been carried out. The patent specification rather reads like a general recipe for the cloning of any cDNA. The screening of the potential recombinant cDNA clones requires that each of them be separately tested for biological activity. In the absence of any step of beta-IFN mRNA enrichment before cDNA cloning as well as of any specific means for selecting full length cDNA molecules and for ruling out the antiviral activity of the bacterial extracts, it must be beyond feasibility. The board thus concludes that the method according to document (89) is not workable and that this document is not relevant to novelty.

20. The novelty of claims 13 and 14 has also been challenged in view of document (66) or (77) which disclosed pure beta-IFN. None of these papers is concerned with making pharmaceutical preparations of beta-IFN. Nor do they provide information on how to make sufficient amounts of the protein for such

preparations. The authors of document (66) (page 706) express their concern that the beta-IFN they isolated was not entirely pure. Document (66) or (77) cannot be damaging to novelty.

21. From all this, it follows that the novelty of the claims of the main request has to be acknowledged.

Inventive step

22. The closest prior art is document (2) which discloses the cloning of beta-IFN cDNA as well as its sequence.

23. Starting from this prior art, the objective technical problem to be solved is the recombinant production of a polypeptide displaying immunological or biological activity of human beta-IFN.

24. Considering that the need for beta-IFN was clearly expressed in the prior art and that recombinant DNA technology was generally regarded as the means to produce a hitherto rare protein (document (7)), the formulation of this problem is obvious.

25. The solution provided in claim 1(a) is to construct recombinant plasmids where the beta-IFN cDNA is inserted downstream of a promoter in such a manner that it would be transcribed from this promoter and translated in an active form.

26. In part C of the specification of the patent in suit, the three specific plasmids of claim 1(a) are shown to direct the synthesis of polypeptides answering the terms of the claim, albeit in small quantities, but "recombinant production" is not synonymous with production at a high level. Thus, the board is satisfied that the above-stated problem has been solved.

27. At the priority date, the recombinant expression of genes from higher eucaryots had already been achieved. Recombinant expression vectors had been constructed for the production of proinsulin, human growth hormone and ovalbumin in a fused state from the p_{amp}, p_{trp} and p_{gal} promoters, respectively (documents (10), (12) and (34). In the same manner, the human growth hormone and SV40 t antigen coding sequences had been linked to the p_{lac} promoter in such a way that both proteins could be produced in an unfused state (documents (22) and (23)). In the board's judgment, all of these achievements imply that the insertion of the beta-IFN coding sequence downstream of a promoter so that it would be transcribed from its promoter and subsequently translated in an active form must prima facie have been considered quite feasible.

28. The opposition division came to the opposite conclusion on the grounds that two groups which achieved expression of beta-IFN cDNA shortly after the respondent did not make use of the readily available vectors (documents (14) and (21)) but of vector systems which had been published after the priority date of the patent (document (53)) or which were not available to the public (document (21)).

29. The vector systems developed in documents (53) and (21) were devised to ensure the in-frame translation of the foreign coding sequence in an unfused state. They lightened the task of the skilled person. In the board's judgment, the authors of documents (14) and (21) were totally justified in using these newer and more efficient tools to reach the goal they had set themselves. Yet, this does not mean that, at the priority date, the skilled person aware of the difficulties inherent in in-frame translation would necessarily have discarded the previously existing vectors. In fact, in document (12) (page 605, third

column, second paragraph) these problems were already acknowledged and the analogous solution was proposed with the p_{trp} expression system and in a more generic manner in document (53) with the p_{lac} promoter system.

Thus, the board concludes that the construction of the beta-IFN expression vector per se, using promoter systems known to work in the prior art should not require more than routine work from the average skilled person. The point which remains to be decided is therefore whether the skilled person would have reasonably expected the beta-IFN cDNA to be expressed in the recombinant host as an active protein, in the light of the known properties of the human beta-IFN (see section XII, chapter "inventive step", paragraph 4).

31. In this context, it has to be borne in mind that "the hope to succeed" should not be misconstrued as "a reasonable expectation of success" (see T 296/93, OJ EPO 1995, 627). In the board's judgment, the former is the mere expression of a wish whereas the latter requires a scientific evaluation of the facts at hand. In the case of gene expression, this evaluation necessitates that the properties of the "expression partners" (the gene to be expressed and its protein product on the one hand, and the recombinant host on the other) be compared.

32. If any one of them has properties which common general knowledge at the priority date would have suggested might be unfavourable to their relationship, it is justified to conclude that the person skilled in the art would have had no reasonable expectation of success.

33. The situation often occurs, however, that no meaningful comparison can be carried out simply because there is not enough available knowledge on both partners. Such a situation must, thus, be assessed in the light of the prior art, as the average person skilled in the art would have done at the priority date.

34. It has to be assumed that the average skilled person would not engage in creative thinking (see T 500/91 of 22 September 1992). Yet he or she can be expected to react in a way common to all skilled persons at any time, namely that an assumption or hypothesis about a possible obstacle to the successful realisation of a project must always be based on facts. Thus, in the board's view, an absence of evidence that a given feature might be an obstacle to carrying out an invention would not be taken as an indication that this invention could not be achieved, nor that it could.

35. The respondent has pointed to a number of properties of beta-IFN and beta-IFN cDNA as potential sources of difficulties for expression: the high hydrophobicity of beta-IFN, the presence of an uneven number of cysteine residues in its amino-acid sequence, the existence of two narrowly spaced ATGs at the 5' end of the beta-IFN cDNA, the presence of the rare Ile AUA codon. All of these properties will be considered in turn.

36. The documents forming the state of the art at the priority date and dealing with the hydrophobicity of beta-IFN are documents (81) and (82). Both are studies of the beta-IFN ability to stick to specific ligands on columns and both come to the conclusion that beta-IFN binds to the columns by way of hydrophobic interactions. Document (81) stresses the "much more pronounced apparent hydrophobicity" of beta-IFN compared with alpha-IFN.

37. The respondent concludes from these teachings that beta-IFN would have been expected to bind to the cell membranes and, therefore, to be toxic to the cells or to be undetectable.

38. There is no evidence on file to indicate the extent to which an hydrophobic protein was expected to stick to cell membranes or that this phenomenon, if occurring at all, would have been regarded as a cause for lethality. Linking cell death to hydrophobicity thus amounts to a groundless assumption which cannot be retained in the evaluation of "reasonable expectation of success" (see point 34, supra). As to the problem of undetectability, the solution to this problem is provided by document (82) which discloses that active beta-IFN may be retrieved from a hydrophobic association with the help of ethylene glycol. The board thus concludes that the apparent hydrophobicity of beta-IFN, while known, would not have been perceived by the person skilled in the art as seriously putting in jeopardy its expression in a recombinant host.

39. At the priority date it was known that beta-IFN had an uneven number of Cys residues (3) in its amino-acid sequence. Looking at the state of the art at the priority date with regard to proteins with S-S bridges, it becomes apparent that E.coli proteins, the tertiary structure of which involved S-S bridges, were known. Mammalian proteins with S-S bridges (documents (34) and (41)) had been expressed in E.coli transformed cells (documents (34) and (10)). Recombinant human alpha interferon (document (3)) and rat growth hormone (document (12)) which each contained an uneven number of cysteins (5) had been recovered in an active form. Accordingly, the skilled person aware of the state of the art would not have considered the formation of S-S bridges in E.coli from an uneven number of cysteins and

their maintenance to be likely to decrease the expectation of successfully expressing active beta-IFN in said cells.

40. Turning now to the pre-beta-IFN cDNA sequence, it can be seen that it contains two ATGs, twenty codons apart at its 5' end, one of them being the translation initiation codon. The argument runs that the person skilled in the art at the priority date would not have known what effect the neighbouring internal AUG codon on the m-RNA would have on expression. To the board, this means that no deleterious effects on expression had ever been associated with the presence of this codon in this position. Therefore, this follows the reasoning developed under point 34 (supra) that an assumption without grounds cannot enter the evaluation of reasonable expectation of success.

41. Further, it has been pointed out that the presence of the rare Ile codon in the beta-IFN cDNA sequence would have been felt as a potential barrier to the translation of the beta-IFN mRNA as the presence of rare codons was known to limit protein synthesis. A number of documents published before the priority date disclose the recombinant expression in E.coli of proteins encoded by genes containing rare codons. In particular, human growth hormone had been expressed while its DNA contained the AUA Ile codon (document (12)). Yet again, the board is of the opinion that this feature would not have been thought by a skilled person to interfere with beta-IFN cDNA translation in such a drastic manner as to prevent expression.

42. Finally, the respondent argued that the sum total of all these alledged "concerns" would amount to something which would prevent the skilled person to enter the task of expressing beta-IFN. In points 36 to 41, the

board showed that no single "concern" has a scientific basis. As the sum total of no "concerns" can scarcely amount to more than no "concerns", this argument fails.

43. In summary, the respondent's submission that the known features of beta-IFN would necessarily have been regarded as insurmountable obstacles for its expression in recombinant form, even in the absence of any suggestions in the art that such kind of features was likely to cause problems for expression cannot convince the board. Rather to the board, the skilled person would consider the knowledge of the properties of beta-IFN as an asset in identifying in the light of the state of the art which problems, if any, such properties may cause and which solutions were available. By doing so, the skilled person would come to the conclusion that the properties of beta-IFN were not such as to bar the way to its expression.

44. In view of the above findings (parapraghs 27 to 41), the main request is rejected for lack of inventive step.

Auxiliary request

45. In the auxiliary request maintained by the respondent at oral proceedings (second auxiliary request), the polypeptide of claims 1 and 8 to 12 has been restricted to one of the polypeptides which was already claimed in granted claims 1 and 12 to 16 (ie with the biological activity of human beta-IFN). No objections are raised to these claims under Articles 123(2) and (3) and 84 EPC.

46. In relinquishing a claim to a molecule with the immunogenicity of beta-IFN, the respondent may have avoided potential objections that it would not have required any inventive step to produce fragments of the

protein which would have kept some of the immunogenic determinants characteristic of the beta-IFN whole molecule. It remains nonetheless that the board's reasoning concerning the inventive step of claim 1 of the main request (see points 27 to 44, supra) applies equally irrespective of whether the recombinant molecule is characterised by its immunogenic capacities or its biological activity. The same conclusion is thus reached as in paragraph 43, namely that the subject-matter of claim 1 of the auxiliary request fails to fulfil the requirements of Article 56 EPC.

47. The auxiliary request is rejected for lack of inventive step.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked

The Registrar: The Chairwoman:

D. Spigarelli U. Kinkeldey

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